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Multimission Metagenomics Technology Development for Planetary Protection

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Overview

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  - Molecular Biology Methods
- Shotgun Metagenomics
- Project Objectives
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Planetary Protection Context

Planetary Protection aims to preserve the integrity of space exploration by preventing forward and backward biological contamination. Missions to celestial bodies that may have once held an environment suitable for life (e.g., Mars and outer planet icy bodies) have more stringent Planetary Protection requirements.

	Mars 2020	Outer Planet Icy Bodies	Mars Sample Return Concepts	Planning for Humans to Mars
PP Requirements	Microbial Quota	Probability of Contaminating a Liquid Body	Risk of forward and backward biological contamination and knowledge capture	Robotic mission requirements & provisions for human health
Metric Value	$<5x10^5$ at launch	$<10^{-4}$	<1 viable organism/sample tube <1 unsterilized Mars organism	Ongoing COSPAR workshops[1]
Organism Types Considered	Bacterial Spores	All	All	All

[1]COSPAR Working Meeting on Refining Planetary Protection Requirements for Human Missions to Mars

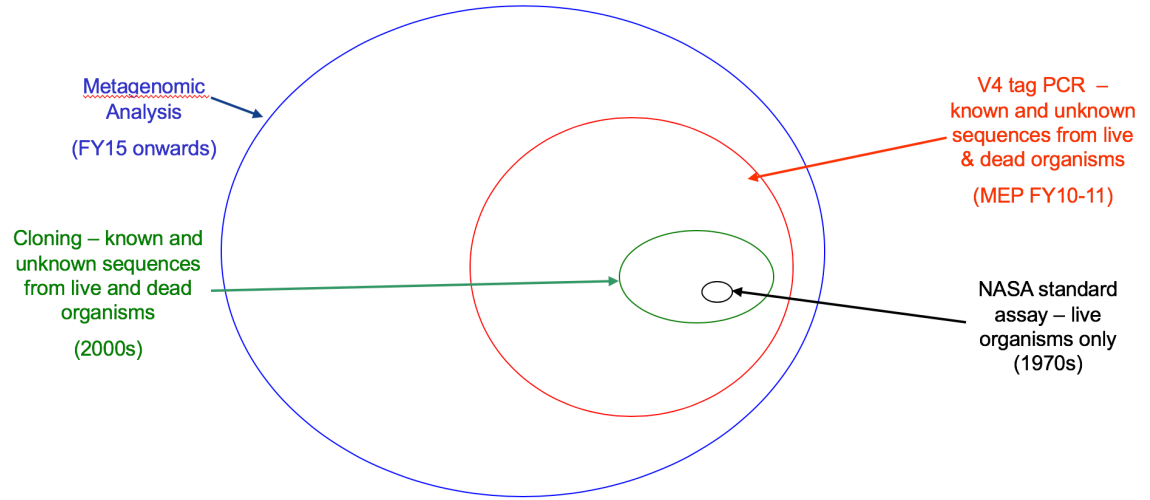
Current Implementation Methods

	Mars 2020	Outer Planet Icy Bodies	Mars Sample Return Concepts	Planning for Humans to Mars
PP Requirements	Microbial Quota	Probability of Contaminating a Liquid Body	Risk of forward and backward biological contamination and knowledge capture	Requirements TBD
Organism Types Considered	Bacterial Spores	All	All	All

- NASA Standard Spore Assay
  - Does not provide information on total microbial populations
  - Selects for cultivable spores; suitable to fulfill spore quota requirements
  - Limited by growth parameters
- < 1 - 5 % of viable microbial population is cultivatable (Jones 1977, Torsvik et al. 1990, Wagner et al. 1993)
  - Smaller subset of that are spores

Molecular Biology Methods

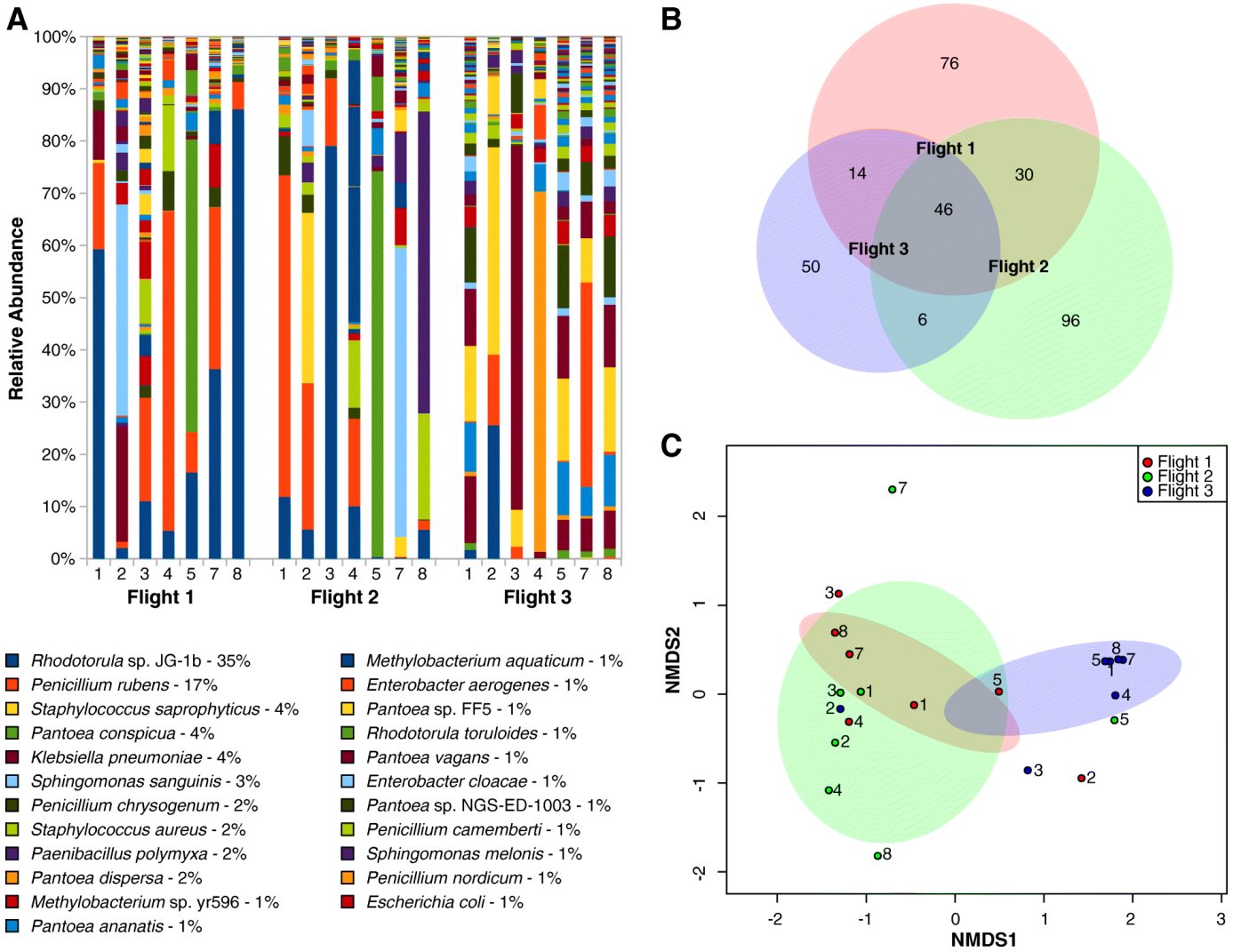
- Need more sensitive assays for the detection of microbial bioburden and for science investigations on complex missions
- Molecular biology methods can provide a modern approach to NASA's current bioburden measurement methods
- Mars Program Office has been working on spacecraft associated DNA analysis since 2000's.
- Mars Program Office currently using taxonomic metagenomics (Genetic Inventory) for biological contamination control knowledge capture for sample return.



Anticipated inventories: metagenomic analysis > V6 tagging (thousands) > classical cloning/seq (circa 100) > cultivation (<100).

Shotgun Metagenomics

- Able to provide profile of microbial community taxonomy
- Can be used to infer microbial community genetic functionality
- Can uncover novel diversity
- Has been used to support ISS research
  - 2018 study on ISS microbial diversity and community dynamics
- Can support and enable future missions by providing a technical basis for the broadest spectrum of organisms to feed into Microbial Risk Analysis Models using taxa and functional identifications



Singh et al. 2018

Objectives of Multi-mission Metagenomics

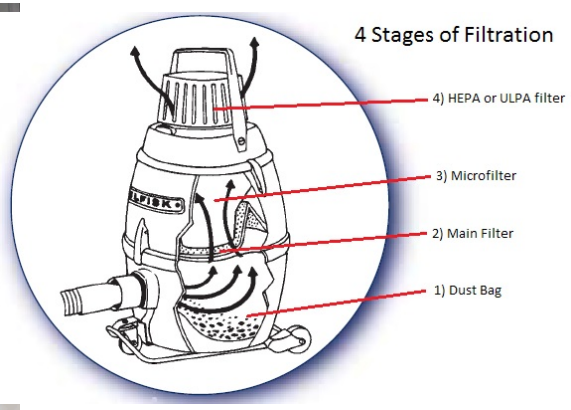
To evaluate the feasibility of a comprehensive tool for the quantification, identification and functional assessment of microbes on spacecraft associated surfaces to supplement/replace the NASA Standard spore-based Assay

- Assess cleanroom biological control capabilities for a standard controlled ISO 7 (ATLO) and a stringently controlled ISO 5 (subsystem assembly).
- Evaluate the potential to provide quantitative inputs into Risk Analysis Models
- Compare 3 external vendors for processing workflow

Technology Development

Sample Collection

Facilities (ultra low)



Vacuums (high)

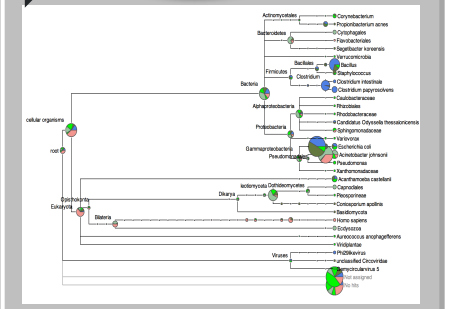
Flow bench filters (low)

Sample Processing

DNA Extraction	Genomic Library Prep/Sequencing	Data Analysis
Optimization and standardization of operating protocols for low biomass environments established.		• Bioinformatic pipeline

DNA → Archive

Raw Data → Archive



Challenge is to establish optimized protocols to produce high quality data from low-biomass samples (10^{-9} g to below detection limit amounts of DNA)

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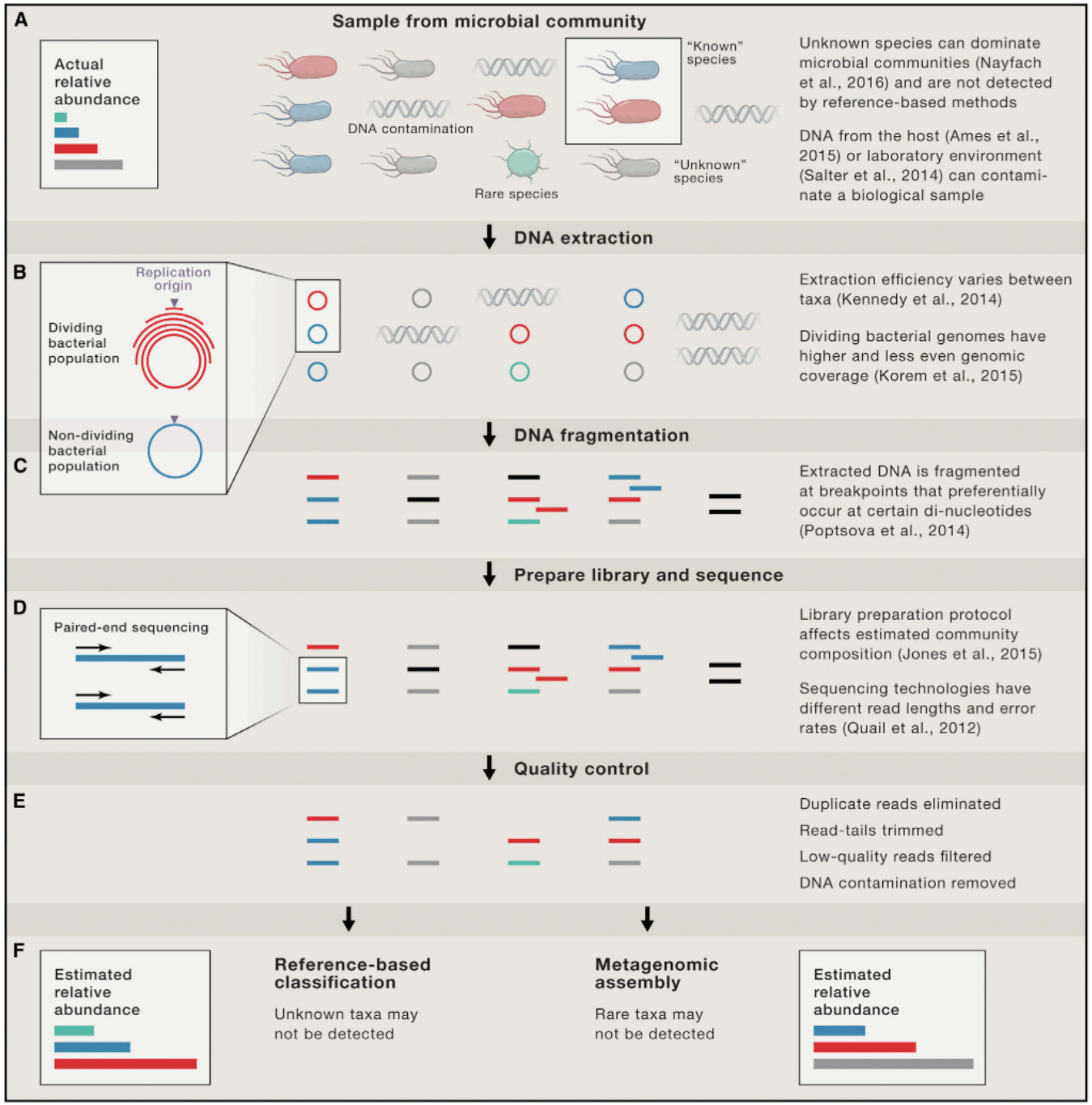
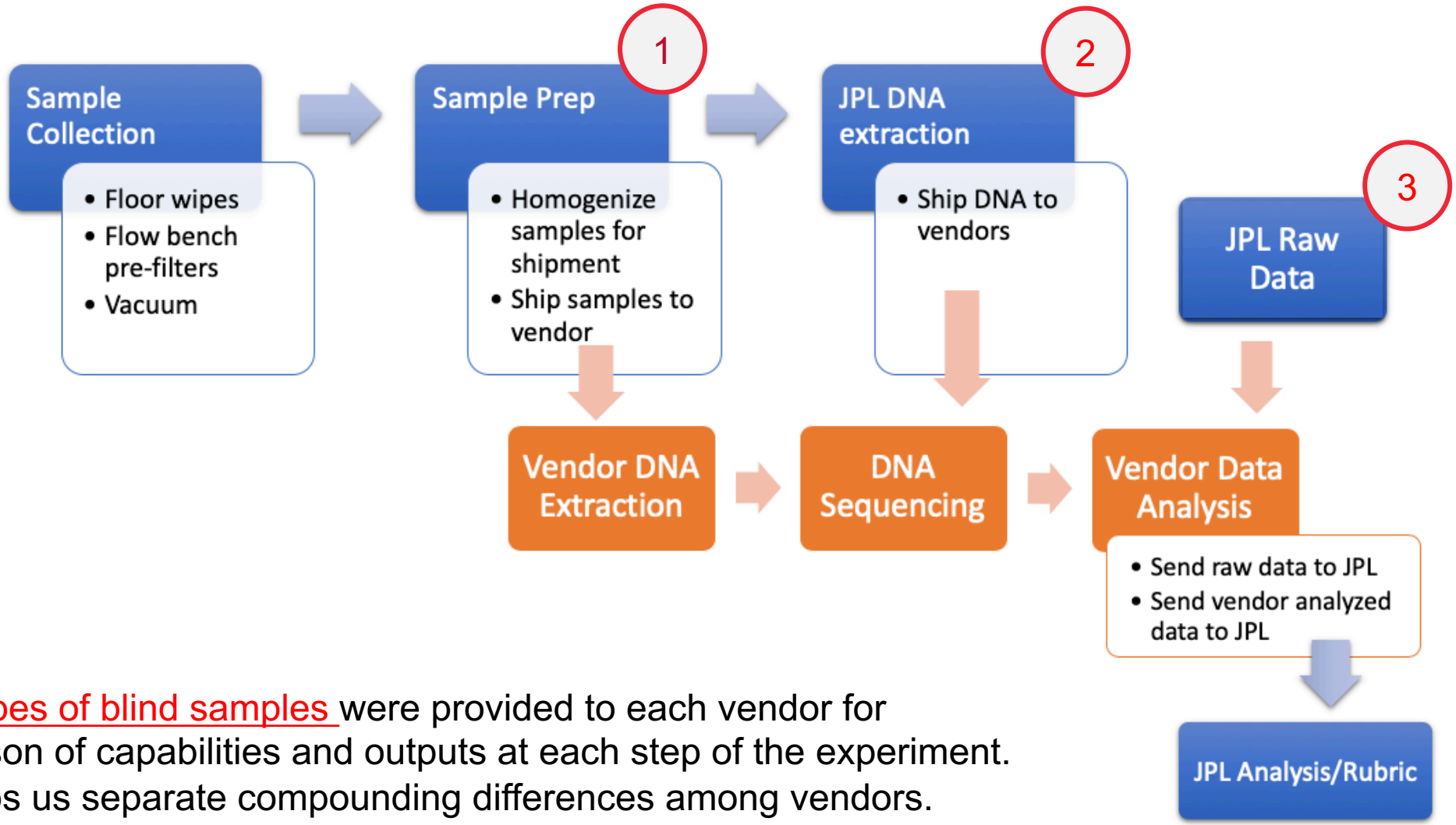


- Differences among vendors are expected
- All steps are contributors to bias
- How can we mitigate bias to capture meaningful information?
- Are effects of bias relevant in scale to biological variation?
- In recent human microbiome studies, *"most experimental biases examined to date are not large enough to obscure biological effects"* (Nayfach et al. 2016, Voight et al. 2015, Lozupone et al. 2013)
- There are consortiums (i.e. Microbiome Quality Control project) dedicated to assessing technical variation in metagenome pipelines.

Nayfach et al. 2016

Experimental Design



Three types of blind samples were provided to each vendor for comparison of capabilities and outputs at each step of the experiment. This helps us separate compounding differences among vendors.

Experimental Design

Cat	Description	ISO 7	ISO 5	Controls	Total # of samples
1	Wipe/Flow bench DNA (extracted by JPL)	5	5	2	12
2	Wipe Samples (extracted by vendor)	5	5	2	12
3	ISO 5 Flow Bench particulates (extracted by vendor)	5	4	1	10
4	Vacuum particulates (extracted by vendor)	5	4	1	10
5	Repeatability (DNA from one sample extracted by JPL)	5	-	1	6
	Total # of samples for shotgun sequencing				50
6	Sequence analyses pipeline check (raw data from JPL)	20	-	-	20

1. All 50 samples should be analyzed using vendor established library preparation, sequencing, and sequence analysis pipeline.
2. As specified in Category 6, JPL will provide raw shotgun data generated from JPL samples (n=20; >1M raw reads per sample) and request the vendor/institution to apply their metagenomics analyses pipeline
3. Vendors will be required to provide negative and positive sequencing control to show sample normalization and sequencing quality

Experimental Design

- Samples collected for cleanroom assessment
  - 20 samples collected from ISO 7 environments
  - 18 samples collected ISO 5 environments
  - Samples (wipes, filters, vacuum bags) were suspended in stabilizing buffer prior to distribution to vendors and in-house extraction.
    - To improve homogenization of samples
  - Extracted DNA concentrations were quantified via Qubit
    - DNA concentrations ranged from below detection limit (wipes) to ~120ng (flow bench filters)

- All raw samples and DNA samples are currently undergoing processing at vendor locations

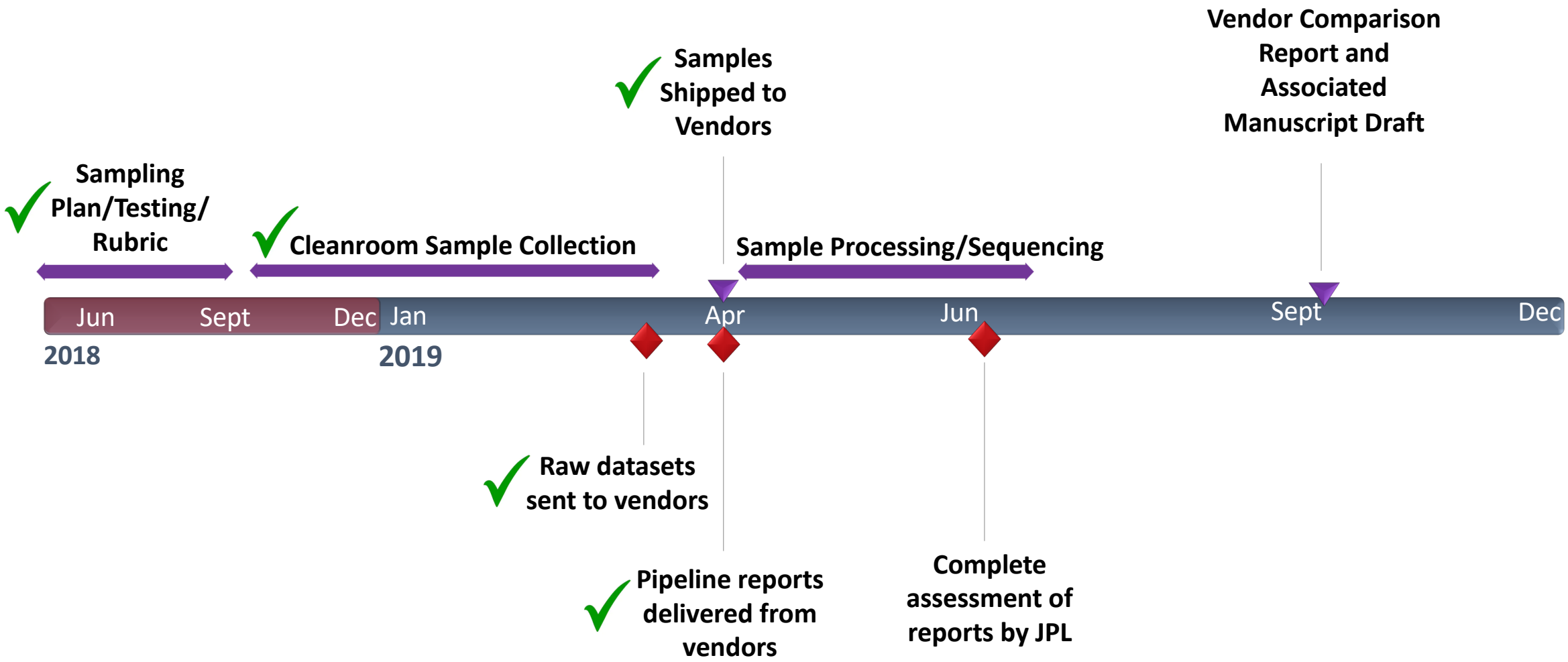
Output Requirements for Vendors

- Metagenomics-based approach for relative quantification of microbial population in cleanroom samples
  - DNA quantification
  - Quality control (raw reads to quality reads)
  - Relative abundance of microbial population
  - Phylogenetic: domain level to species
  - Various types of microbes as defined by below:
    - All known microbes (bacteria, archaea, fungi); alpha diversity
    - As per the Task Group on the Forward Contamination of Europa Space Studies Board's "Preventing the Forward Contamination of Europa" (2000):
      - Type A: Bacteria grown in nutrient rich media (e.g: Tryptic Soy Agar), aerobic, 32˚C, 72-hr growth.
      - Type B: Aerobic spore-formers
      - Type C: Aerobic spore-formers that are resistant to radiation
      - Type D: Non-spore formers that are radiation resistant
  - Functional annotation
    - Radiation resistant genes
    - Spore forming
    - Desiccation resistant

Preliminary Results

- Bioinformatic Analysis: Category 6
  - 71 million reads
  - 20 SAF samples
- Taxonomic analysis from vendors show that majority of taxa identified overlapped among vendors
  - Proportion of overlap higher at genus-level vs species-level
  - Overlap is attributed to dominantly abundant taxa
  - Rare taxa contribute to differences between vendor analysis
  - Quality control parameters have been found to be one of the greatest sources of bias in bioinformatic pipelines (Sinha et al. 2015)

Task Timeline



Future Work

- FY20 and beyond:
  - Engagement of stakeholders outside of NASA – workshop and/or continued publications
  - Continue to refine Standard Operating Protocols (SOP)
  - Spatial and temporal characterization of ISO 7 cleanrooms at JPL
  - Expansion of cleanroom characterization to other critical cleanrooms
  - Validation of SOP using current biological spacecraft builds (e.g. Europa Clipper and Mars Sample Return Concepts)
- Improving throughput rate
  - Robust DNA collection and extraction
  - Accurate, inexpensive long-read sequencers that process overnight
  - Standardization of bioinformatics pipeline to provide compliance status in hours

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